Journal of Veterinary Research Advances
ISSN: 2582-774X

Research Article
Open access

Variation of haemoglobin polymorphism of indigenous cattle

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Received on: 13/08/2025 Accepted on: 17/12/2025 Published on: 20/12/2025

ABSTRACT

Aim: The main purpose of the study was to evaluate the haemoglobin genotyping of two cattle breed wadara and kuri in Nigeria.

Method and materials: A total of 20 matured and healthy cattle was used for the study comprising male and female from two breeds of indigenous cattle (10 Wadara and 10 kuri) respectively. The study therefore assessed the distribution of haemoglobin genotypes and gene frequencies in indigenous cattle breeds. Blood samples (5 mL) were collected from the cattle at slaughter point upon collection, the blood was immediately transferred into EDTA bottles to prevent coagulation and analysed using cellulose acetate electrophoresis to identify the haemoglobin variants present.

Results: Result showed that three haemoglobin genotypes (HbAA, HbAB and HbBB) were detected in both breeds. In Wadara genotype counts were AA = 2 (20%), AB = 5 (50%) and BB = 3 (30%) giving allele frequencies of HbA = 0.45 and HbB = 0.55. In Kuri genotype counts were AA = 4 (40%), AB = 2 (20%) and BB = 4 (40%) with allele frequencies HbA = 0.50 and HbB = 0.50. Observed heterozygosity was higher in Wadara (Ho = 0.50) than in Kuri (Ho = 0.20) while expected heterozygosity was 0.45 and 0.50 respectively. Chi-square tests showed no significant deviation from Hardy–Weinberg expectations for either breed ($p > 0.05$). Genetic distance between the breeds at the haemoglobin locus was very small ($d \approx 0.027–0.009$) indicating close genetic relationship.

Conclusion: It was concluded that baseline data on haemoglobin genotypes and allele frequencies can inform future breeding and conservation decisions for indigenous cattle in northeastern Nigeria.

Keywords: Haemoglobin, Genetic distance, Gene frequency, chi square and Herdy-weinberg equilibrium (HWE).

Cite This Article as: Adamu J, Chana ZM, Ali Marlene Mai and Yakubu RN (2025). Variation of haemoglobin polymorphism of indigenous cattle. J. Vet. Res. Adv., 07(02): 78-83.

Introduction

Nigeria is endowed with varied ecological zones with diverse animal genetic resources of local breeds. These local breeds possess genes relevant for their survival and adaptation to environment and local breeding goals. According to Dossa and Vanvanhossou (2016) local cattle are poor milk and meat producers as compared with their exotic counterparts though these are better adapted to survive and tolerate harsh environment (Hirwa et al., 2017). Indigenous cattle breeds have unique morphological features which distinguishes them from other cattle. These include horn shape and size (Ndumu et al., 2008; Kugonza et al., 2011). The characterization of livestock at genetic and biochemical level is critical to understanding population structure, adaptation and evolutionary relationships.

One of most informative tools is analysis of blood protein polymorphisms such as found in haemoglobin (Hb), transferrin (Tf) and carbonic anhydrase (CA). These biochemical markers serve as indicators of genetic variability and can be used to distinguish between breeds, measure genetic distance and guide conservation and breeding strategies (Ibeagha-Awemu and Erhardt, 2004; Salako and Ngere, 2002). Haemoglobin polymorphism in particular has been extensively used in cattle genetic studies due to its codominant expression and simplicity of detection through electrophoresis. Variants such as Hb^A and Hb^B represent different alleles at the haemoglobin locus and their frequency distributions can provide insights into genotype-environment interactions especially in thermally stressful or disease-prone environments (Ibrahim and Alade, 2018; Yakubu and Salako, 2009). Haemoglobin is the iron-containing protein in red blood cells responsible for transporting oxygen. In cattle, two common allelic forms at the haemoglobin locus are Hb-A

and Hb-B resulting in three genotypes: Hb-AA, Hb-AB and Hb-BB. The distribution of these genotypes varies between breeds and can be used to determine allele frequencies, assess population structure and estimate genetic distances (Ojo et al., 2014; Egahi et al., 2010). Understanding the distribution of haemoglobin genotypes and other biochemical variants enables researchers to calculate gene and genotype frequencies which are foundational to measuring genetic distance(a quantitative estimate of divergence between breeds or populations). Genetic distance is crucial for assessing how genetically similar or distinct populations are especially when evaluating the risk of inbreeding, the need for conservation or the potential for crossbreeding programs (Nei, 1972; Weir and Cockerham, 1984). The general objective of the study was to evaluate the haemoglobin genotyping of two cattle breed.

Materials and Methods

The study was conducted at Maiduguri Central Abattoir, the largest abattoir in Northeastern Nigeria, located in Maiduguri, Borno State (11°50′42″N, 13°09′36″E; elevation ~325 m). The area lies within the Sudan–Sahel savanna, characterized by a semi-arid climate with mean annual rainfall of about 519 mm (range 292.7–838.2 mm) and temperatures generally between 25°C and 40°C (Abatcha et al., 2024). The abattoir processes up to 200 cattle daily, along with sheep and goats, serving as a key center for meat supply and livestock disease surveillance in the region (Ghide et al., 2024).

Experimental Animals: A total of 20 matured and healthy cattle was used for the study comprising male and female from two breeds of cattle(10 wadara and 10 kuri) respectively. The animals were randomly selected at the maiduguri abbatoir cattle market (kasuwan shanu). The animals were brought from different parts of the state for sales and slaughter.

Data Collection: About 5ml of blood sample was collected at the point of slaughter from 10 wadara and 10 kuri breed of cattle and was used for the study. The collection was done between 7am to 9am. Upon collection, the blood was immediately transfer into EDTA bottles to prevent coagulation and was put in ice pack before taking to the laboratory for Analysis. The analysis was conducted at the Department of Animal Science Laboratory.

Laboratory Analysis: About 0.5 to 1ml of whole un sedimented blood was drawn into a vacutainer tube and 10- 15ml of cold NaCl (0.155ml.) was added to wash the red blood cells, the samples were centrifuged at 50C for 5 minutes at 3000 r.p.m. the supernatant was discarded and distilled water was added to the sediment cells to release the haemoglobin by hemolysis. A transfer pipette was used to remove the hemolysate, a cellulose acetate strips was prepared and labelled with pencil. The strips was later soaked in Tris-EDTA borate buffer (TEB, pH 8.6) and blotted lightly with a filter paper. Haemolysate was applied with a micropipette at the cathode and electrophoresis was performed at pH 8.6 using a Shandom Southern Electrophoresis tube with TEB as electrode buffer at 100V for 15-20 minutes. The strips was later removed and stained with Poncean Red Stain for 5-10 minutes. The strips was later de-stain first in 5% ethanoic acid (acetic acid) followed by 12% ethanoic acid (acetic acid) solution until the strips was clear of stain. The strips was later dried in the oven, allelic variants for the haemoglobin (Hb) locus was mark in order of increasing mobility. It was represent as A and B respectively and then scored based on the band following the normal procedure as previously described by (Osaiyuwu et al., 2013).

Statistical Analysis: Allele and genotypic frequencies for the locus in each sample was computed by direct gene counting method. The distribution of the haemoglobin genotypes was tested using Chi-square analysis. The genotype frequency for each of the population was estimated using the following expression (Salako et al., 2007).

$$AA = \frac{\text{No of AAx100}}{\text{No of individual}}$$

$$BB = \frac{\text{No of BB x100}}{\text{No of individual}}$$

$$AB = \frac{\text{No of AB x100}}{\text{No of individual}}$$

The gene frequency for each of the population was estimated using the expression, provide by (Roughgarden *et al.*, 1979)

$$\text{Allele A} = \frac{2nAA+nAB}{N/2}$$

$$\text{Allele B} = \frac{2nBB+nAB}{N/2}$$

Where N = total no of individuals sampled in each population

nAA= Observed genotype number for AA

nBB = Observed genotype number for BB

nAB = Observed genotype number for AB

The genetic distance between Kuri and wadara cattle was estimated according to the method of

Bodmer and Cavalli-sforza (Roughgarden *et al.*, 1979) with the following expression

$d_2 = (P1-P2)_2$

where d_2= genetic distance

P1=gene frequency of allele A

P2=gene frequency of allele B

$$\text{Chi – Squarem}(X_2) = \frac{\Sigma (\text{Observe No.} - \text{Expected No.})_2}{\text{Expected}}$$

Local inbreeding Co-efficient (FS), local observation (L+o) and local expected (L+e) heterozygosity were calculated from the genotypic counts. Expected and observed heterozygosity deficit within FST= genetic differentiation (Fst). Genetic distance matrix was computed from the various gene frequencies of individual cattle breed using Microsoft excel spread sheet Where Xa and Ya are frequencies of alleles drawn from population X and Y (Reynoid et al., 1983) genetic distance dxy.

Results and Discussion

Distribution of Haemoglobin Genotyping and Gene Frequency of Cattle Breeds (Table 1) showed distribution of haemoglobin genotyping and gene frequency of two cattle breeds there exist three types of haemoglobin genotypes (HbAA, HbAB and HbBB) in both Wadara and Kuri cattle. (Naik et al., 1968; Pal and Mummed, 2014) also confirmed the presence of two co-dominant alleles HbA and HbB which give rise to three common genotypes: Hb AA, Hb AB and Hb BB. There was variation or polymorphism in the haemoglobin of these breeds. The Wadara cattle most common genotype was HbAB(50%) followed by HbAA(20%) and HbBB(30%). It means both genotype had HbA (0.45) and HbB(0.55) genes. Having more heterozygote (HbAB) suggests that Wadara cattle have a lot of genetic diversity, which is important for survival and adaptation.

A study on 150 indigenous cattle (Bunaji and Sokoto Gudali) in Northwestern Nigeria was conducted and observed allele frequencies were HbA = 0.62 and HbB = 0.38 with HbAB being most frequent genotype. It was reported no significant deviation from Hardy-Weinberg equilibrium, suggesting a stable gene pool at haemoglobin locus. Observed polymorphism could serve as a valuable genetic marker for characterizing local cattle populations (Oladepo et al., 2022). Haemoglobin genotyping in Kuri cattle revealed HbAA (40%), (HbAB 20%) and HbBB (40%). The two alleles HbA and HbB frequencies were equal (0.50 each) indicating balanced polymorphism and genetic stability at haemoglobin locus. It supported co-dominant inheritance previously reported in cattle (Henkes et al., 2000; Pal and Mummed, 2014). The equal distribution suggested ongoing balancing selection, possibly influenced by environmental adaptation. Earlier, Oladepo and Salako (2017) observed a higher HbB frequency (0.77) in Borno Kuri, attributing it to breed's adaptation to hot and arid climates, consistent with Tsunoda (2006) who linked HbB with thermotolerance and efficient water retention. The presence of both alleles reflected genetic variability that enhances adaptability and aligns with Hardy–Weinberg equilibrium expectations (Nicholas, 2010).

Observed and Expected Genotyping Counts and Chi-Square of Cattle: The observed and expected genotype frequencies in Kuri and Wadara cattle breeds (Table 2) revealed that Wadara cattle observed genotype frequencies (HbAA = 02, HbAB = 05 and HbBB = 03) were very close to the expected frequencies (HbAA = 2.03, HbAB = 4.95 and HbBB = 3.025) calculated under Hardy–Weinberg equilibrium.

Table1: Distribution of haemoglobin genotyping and gene frequency of cattle breeds

Population	Genotype Frequency				Gene Frequency		
	AA	AB	BB	No	A	B	Total
Wadara (WC)	02	05	03	10	0.5	0.5	1.00
Kuri(KC)	04	02	04	10	0.45	0.55	1.00

Table 2: Observed and expected genotypic count and Chi-square for two breeds of cattle

	Population					
	Wadara			**Kuri**		
	AA	**AB**	**BB**	**AA**	**AB**	**BB**
Observed	02	05	03	04	02	04
Expected	2.03	4.95	3.025	2.5	5.0	2.5
Deviation	-0.025	0.05	-0.025	1.5	-3	1.5
Expected Deviation	0.025	-0.05	0.025	-1.5	3	-1.5
χ^2	0.000309	0.000505	0.000207	0.9	1.8	0.9
χ^2 Total	0.00102			3.6		
Tabulated Value	5.99	at 2df		5.99	at 2df	
	*P(>0.05)					

The number of heterozygotes (HbAB = 05) observed was slightly higher than the homozygote (HbAA = 02 and HbBB = 03) which suggests that both HbA and HbB alleles are being maintained in the population. The chi-square value for Wadara cattle ($\chi^2 = 0.001$) much lower than the critical value (5.99) at 2 degrees of freedom. The deviation between observed and expected genotype frequencies was not significant ($p > 0.05$). This means that: Random mating is likely occurring, No strong selection pressure acting against any genotype and there is a stable population with balanced allele inheritance. The Kuri cattle observed genotype frequencies values (HbAA = 04, HbAB = 02 and HbBB = 04) showed noticeable deviation from the expected values (2.5, 5.0 and 2.5 respectively). The heterozygous genotype (HbAB) was lower than expected while both homozygous genotypes were higher. This pattern may suggest reduced heterozygosity within the population which could be associated with a relatively small breeding population or limited mating opportunities. However, the chi-square analysis indicated that this deviation was not statistically significant ($\chi^2 = 3.60$, $df = 2$, $p > 0.05$), as the calculated value was lower than the tabulated value of 5.99. This implies that the differences between observed and expected genotype frequencies might have occurred by chance and do not represent a true genetic shift in the population. Therefore, the Kuri cattle population does not significantly deviate from Hardy–Weinberg equilibrium for haemoglobin genotype distribution. Although slight variation exists in genotype frequencies, there is insufficient statistical evidence to conclude the presence of strong selective pressure, genetic drift or marked inbreeding. Continued monitoring may still be beneficial to ensure long term genetic stability and conservation of this indigenous breed.

Local Observed, Expected and Inbreeding co-efficient, Heterozygosity Indices and F- Statistics for Cattle Breeds: it was showed the genetic diversity parameters for the local observed, expected and inbreeding coefficient revealed notable differences between the Wadara and Kuri cattle populations (Table 3). The observed heterozygosity (Ho) was higher in Wadara (0.50) than in Kuri (0.20) which means 50% of the Wadara individuals are heterozygote while Kuri only 20% are heterozygotes. The expected heterozygosity (He) values were 0.45 and 0.50 for Wadara and Kuri respectively. Wadara (0.45) was close to the observed value (0.5), suggesting equilibrium. Kuri (0.5) was much higher than the observed (0.2) showing reduced heterozygosity possibly due to inbreeding or genetic isolation. The fixation index within subpopulations (Fs) was negative in Wadara (-0.505) implying an excess of heterozygotes and random mating. Conversely, the positive Fs value in Kuri (0.6) reflects a deficit of heterozygotes and possible inbreeding. The total heterozygosity (HT) was 0.2025 for Wadara and 0.25 for Kuri suggesting moderate overall diversity across both populations. The inbreeding coefficient within individuals relative to their subpopulation (FIS) was -0.1111 for Wadara confirming the absence of inbreeding while a high FIS of 0.6 in Kuri reveals substantial inbreeding pressure. Similarly, the genetic differentiation among subpopulations (FST) was very high in Wadara (0.909) but absent in Kuri (0.0) implying that Wadara is genetically distinct while Kuri shows uniformity, possibly due to isolation and restricted gene flow. The overall inbreeding coefficient (FIT) followed a similar trend, with near-zero value in Wadara (-0.0101) and high value in Kuri (0.6). These results collectively indicated that Wadara cattle possess greater genetic variability and are genetically stable whereas Kuri cattle exhibit reduced heterozygosity and higher inbreeding tendencies emphasizing the need for genetic monitoring and conservation measures.

Table 3: Local, observed, expected and inbreeding coefficients, heterozygosity indices and F- Statistics for cattle breeds

Parameters	Population	
	Wadara	kuri
Ho	0.5	0.2
He	0.45	0.5
Fs	-0.505	0.6
HT	0.2025	0.25
FIS	-0.111111	0.6
FST	0.90909	0
FIT	-0.0101	0.6

Ho = Observed Heterozygosity, He= Expected Heterozygosity, Fs= Fixation Index within Sub Population, HT = Total Heterozygosity, FIS = Inbreeding Coefficients within Individual Relatives to their Sub Population, FST = Genetic Differenciation among Sub Population, FIT = Overall inbreeding Coefficients.

Genetic Distance between Two Cattle Breeds: The genetic distance values obtained for the wadara and kuri at the haemoglobin loci (Table 4) revealed very low levels of differentiation between the breeds.

As expected, the within-breed distances were zero indicating that animals within each population were genetically similar at these loci. However, the distance between Wadara and Kuri (0.02698 and 0.009165) was also very small, showing that the two breeds share a close genetic relationship.

Table 4: Genetic distance between two cattle breed

	Wadara	Kuri
Wadara	0.000	0.02698
Kuri	0.009165	0.000

According to Nei's classification, values below 0.05 indicate little or no meaningful genetic separation. This suggests that Wadara and Kuri cattle may have originated from a related ancestral population or have experienced gene flow over time. According to Bruchi et al.(2003) the rate of gene flow between populations determines how genetic diversity is distributed both within and between populations. They further reported that extent of gene flow in a population depends on distribution of the habitat it occupies and the size and degree of isolation.

Conclusion

The results showed that both breeds possess the three haemoglobin genotypes (HbAA, HbAB and HbBB), although the distribution varied between them. Wadara cattle had a higher proportion of heterozygotes (HbAB) which also reflected in their higher observed heterozygosity (0.50), In contrast, Kuri cattle showed a more balanced distribution of homozygous genotypes (HbAA and HbBB), resulting in lower heterozygosity (0.20). The allele frequencies revealed that both breeds carry the HbA and HbB genes at relatively similar levels with Wadara showing a slight dominance of HbB and Kuri displaying equal frequencies for both alleles. The Hardy–Weinberg equilibrium test indicated that genotype distributions in both breeds did not significantly deviate from expected proportions suggesting that neither population is under strong selection pressure at haemoglobin locus. Furthermore, the genetic distance between two breeds was very small indicating a close genetic relationship and suggesting that they may share a common ancestral background or have experienced gene flow over time. Overall, the study provides valuable baseline information on the haemoglobin genetic profiles of Wadara and Kuri cattle. Understanding these patterns is important for future breeding, conservation and improvement programs especially as both breeds play significant roles in livestock systems of northeastern Nigeria.

Reference

Bruchi A Marti O and Ritz L (2003). Gene flow and genetic structure in livestock. Animal Genetics, 34(3): 190–198.

Dossa LH and Vanvanhossou FU (2016). Cattle genetic resources in West Africa. Animal Genetic Resources, 58: 98–109.

Egahi JO, Dim NI Momoh OM and Gwaza DS (2010). Haemoglobin variation in White Fulani and Sokoto Gudali cattle. Electronic Journal of Animal Science, 2(1): 10–15.

Ghide AA, Abdu PA and Saidu SN (2024). Slaughterhouse surveillance in Borno State. Nigerian Veterinary Journal, 45(1): 12–22.

Henkes LE, Jolly RD and Coup MR (2000). Haemoglobin polymorphism analysis. Journal of Animal Breeding, 34(1): 45–52.

Hirwa C, Ndahetuye JB and Shabtay A (2017). Adaptability of African cattle breeds. Tropical Animal Science Journal, 40(2): 122–131.

Ibeagha-Awemu EM and Erhardt G (2004). Cattle diversity using microsatellites. Animal Genetics, 35(2): 117–125.

Ibrahim M and Alade A (2018). Haemoglobin alleles in Nigerian zebu. African Journal of Animal Science, 5(1): 26–34.

Kugonza DR, Nabasiige Mn and Hanotte O (2011). Morphological characterization of indigenous cattle. African Journal of Animal Breeding, 6(3): 55–66.

Naik SN, Bhatia HM and Sanghvi L D (1968). Haemoglobin variants in Indian zebu cattle. Indian Journal of Animal Genetics, 2(1): 33–38.

Ndume DB, Baumung R, Jianlin H, Kibogo H and Solknar J (2008). Genetic and Morphological characterisation of the Ankole longhorn cattle in Africa Greatlakes region. Genetic Selection and Evolution 40(5): 467 doi: 10.1186/1297 -9686-40-5-467

Nei M (1972). Genetic distance between populations. The American Naturalist, 106(949): 283–292.

Nicholas FW (2010). Introduction to veterinary genetics (3rd ed.). Wiley-Blackwell.

Ojo VOA, Akinyemi MO and Ojedapo LO (2014). Haemoglobin types and physiological traits. Journal of Animal Science Advances, 4(7): 944–950.

Oladepon O and Salako AE (2017). Blood protein polymorphism in Nigerian cattle breeds.

International Journal of Animal Genetics, 5(2): 99–108.

Oladepo O, Salako AE and Yakubu A (2022). Haemoglobin variation in indigenous cattle. Nigerian Journal of Genetics, 36(1): 55–64.

Osaiyuwu OH, Okoh AE and Akpa GN (2013). Electrophoretic detection of haemoglobin types. Nigerian Veterinary Journal, 34(2): 23–31.

Pal SK and Mummed YY (2014). Haemoglobin polymorphism in Ogaden cattle. Veterinary World, 7(4): 229–233.

Reynolds J Weir BS and Cockerham CC (1983). Estimation of genetic distance. Genetics, 105(3): 767–779.

Roughgarden J (1979). Theory of population genetics and evolutionary ecology. Macmillan.

Salako AE and Ngere LO (2002). Blood protein markers in cattle. Nigerian Journal of Animal Production, 29(2): 24–32.

Salako AE, Ngere LO and Ojedapo LO (2007). Gene frequency estimation in Nigerian cattle. Tropical Animal Science Journal, 40(3): 155–161.

Weir BS and Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38(6): 1358–1370.

Yakubu A and Salako AE (2009). Haemoglobin variation in Zebu cattle. Nigerian Journal of Animal Science, 11: 55–62.

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